RESEARCH ARTICLE

Bioformulation of Animal Waste and Plant Extracts for Degradation of Pesticides

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ABSTRACT: Due to human activities the environment gets polluted and hazardous to the human health. Pesticides are the biological agent mainly used in the agriculturural field to control pests, weeds, insects for better yield production. Organophosphrous is one of the important insecticide which is used all over the world which is very harmful to all the organisms present in the ecosystem. Phytodegradation is the technique which is transformation of toxic contaminants into non toxic with the help of plants. Prosopis juliflora is the evergreen tree grown in exhaustic climatic conditions rich in antimicrobial activity. The degradation of dimethoate insecticide with leaf of *prosopis juliflora* with microbes like *salmonella sp* along with goat dung shows better degradation when compare to other methods.

KEYWORDS: *Prosopis juliflora* , Organophosphrous, Phytodegradation

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1. INTRODUCTION

Agriculture is considered as the backbone of India. More than 60 – 70% of people, mainly depend upon the agriculture. India is one of the country with a fast growing population to cross 1.3 billion by 2020 (1). To overcome the population the country produces enough food for the people. To get high yields the farmers use pesticide to control the pest. Pesticides are the group of artificially synthesized substance that are non biodegradable, toxic to the environment and persist in the ecosystem for a long period of time (2). According to the mode of the action or by chemical function the pesticides are classified as insecticide (insects), fungicide (fungi), rodenticide (rodents), moluscicide (snails), defoliant(leaf harvesting),dissecting(foliage).More than 500 different types of pesticide used in the environment for agricultural activities. Mainly pesticides considered as the contaminant because 1-2% of pesticide reaches the target remaining accumulate in the environment. It reaches all over the ecosystem and affects air, surface and ground water. According to WHO, approximately 3 million peoples are intoxicated each year as a result of using the pesticides. (3). Pesticide cause cardiopulmonary, neurological, skin disorder, fetal deformities, miscarriages, lower the sperm count, etc. (4). Dimethoate is the group of organophosphrous (OP) insecticide that are used to kill mites and insects.(5). In the year of 2010, 950 metric tons of dimethoate were produced and 636 metric tons were used all over the world. (6). The half life of dimethoate is 4-16 days. (7).

Phytoremediation is the most important technique to remove the pollutants from the environment by using plants. Phytotransformation is the plants transform organic contaminants into less toxic, less mobile or more stable form. This process also called phytodegradation, which is the metabolism of the organic contaminants by the plant enzymes.

Prosopis juliflora is one of the important medicinal plant and important sources of antibacterial, antifungal and antioxidant compounds. Prosopis juliflora is mesquite tree belongs to fabaccae family. The phytochemical and antibacterial properties of ethanolic leaf extract from this tree act against ten bacterial isolate and an antimicrobial remedy.

The present study aims to enhance the estimation of secondary metabolites and phytoremediation of pesticides by using Prosopis juliflora along with goat dung.

2.MATERIALS AND METHODS

SAMPLE COLLECTION

Leaf Collection

The leaf of Prosopis juliflora was collected near Erode, Tamilnadu, India and the leaves were thoroughly washed in tap water and rinse several times by distilled water and they were a shade dried and taken for further analysis.

Leaf Crude Extract Preparation

The dried leaves were powdered in a blender and used for extraction. The extract was prepared using ethanol. The crude extracts were used to perform quantitative phytochemical analysis and antibacterial activity.

3. QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *PROSOPIS JULIFLORA*

Determination of Flavanoids

10g of the sample was extracted with 150 ml of 80% aqueous methanol at room temperature The extract was filtered using Whatman filter paper no. 42.The filtrate was transferred into a dish, evaporated to dryness and weighed for ferther use.

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Determination of Alkaloids

The 0.5g plant powder was dissolved in 10%acetic acid in methanol (20ml).This was kept as such for 4hours. Then it was filtered and the extracts were kept in a water bath ¼ of its original volume. Concentrated ammonium hydroxide was added to drop wise to the extract and precipitated. The precipitated washed with ammonium hydroxide then filtered and alkaloid collected in the form of residue (8)

Determination of Perpenoids

10g of plant powder were taken separately and soaked in alcohol for 24 hours, filtered. The filtrate was extracted with petroleum ether and the ether extract was treated as total terpenoids. The residue obtained was dried and weighed. (9)

Determination of Tannis

About 500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1hour in the mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of filtered was a pipette out into a test tube and mixed with 2ml of 0.1M $Fecl_3$ in 0.1 Hcl and 0.008M potassium ferrocyanate. The absorption was measured at 730nm within 10 minutes. The standard graph was plotted using tannic acid in different concentration. (10).

Determination of Phenolics

Total phenol was determined by folincio-calteu reagent. A dilute extract of plant extract or Gallic acid was mixed with folincio-calteu reagent and aqueous NA_2CO_3. The mixture was allowed to stand for 15minutes and the total phenols were determined by calorimetry at 765nm.

4. ANTIBACTERIAL ACTIVITY BY DISC DIFFUSION METHOD

Antibacterial activity with four different concentrations of leaf extracts were tested by agar well diffusion method (11). The bacterial strains were inoculated in 100 ml nutrient broth and kept incubation for 24hrs at 37º C. Muller Hinton agar was prepared and poured into petri dish and inoculated with the test organism by using cotton swabs. Width of six millimeters of sterile discs had been impregnated with 20µl of test extract and introduced on the agar plate. The plates were incubated overnight at 37ºC. Antibacterial activity was assigned by a zone of inhibition around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10µg/disc) and penicillin (10µg/disc) were used as standards.

Plant Analysis

In the laboratory, plant samples were rinsed with tap water to remove firmly attached soil particles from the leaves, stem and root. The samples were then rinsed with distilled deionized water. A stainless steel knife was used to cut the plant samples into different parts (shoot and roots). Plant samples were air-dried for two weeks at room temperature by spreading them on nylon fabric, followed by oven drying at 70°c for 48 hours. Thereafter the samples were ground using a mortar pestle.

5.DEGRADATION OF DIMETHOATE IN *PROSOPIS JULIFLORA* ALONG WITH GOAT DUNG

About 5ml of Dimethoate was added in chloroform, in control tube. The working standard for Dimethoate (0.1ml in 1li water).5ml of dimethoate was added in 0.1ml of Goat dung (10^{-4}) in first tube. 5ml of Dimethoate was added in 0.1mg of plant extracts was added in the second tube. 5ml of Dimethoate was added in 0.1mg of plant extracts and added in 0.1ml of Goat dung (10^{-4}) was added third tube. 5ml of Dimethoate was added in 0.1gm of plant extracts and added 0.1ml of Goat dung (10^{-4}) was added in fourth tube. 5ml of Dimethoate was added in 0.1gm of plant extracts and 0.1ml of Goat dung (10^{-5}) was added in fifth tube. Then take the OD value.

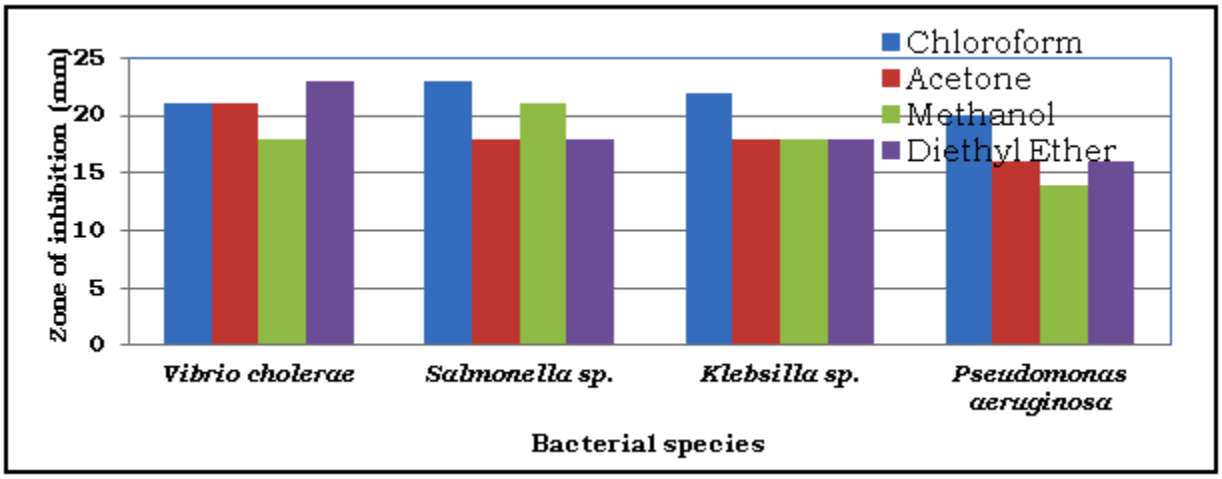

Figure 1: Antibacterial Activity of *Prosopis Juliflora* Leaf Extracts

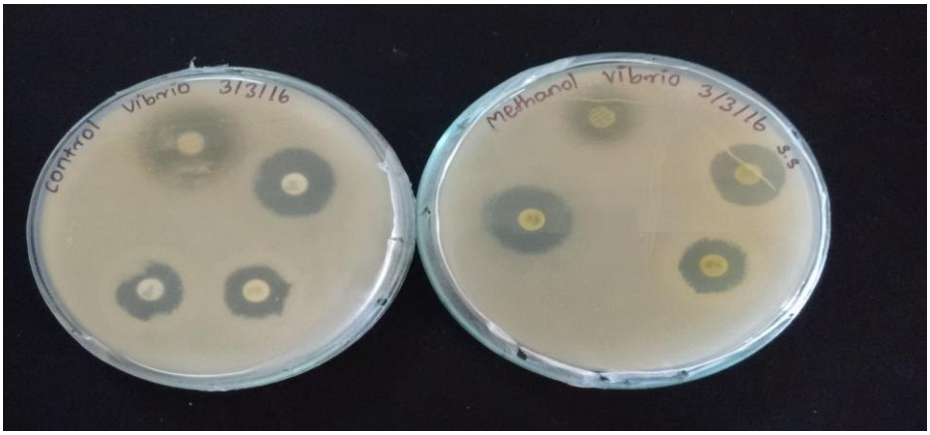

Plate 1: Zone of inhibition of methanol extract in *vibrio sp*

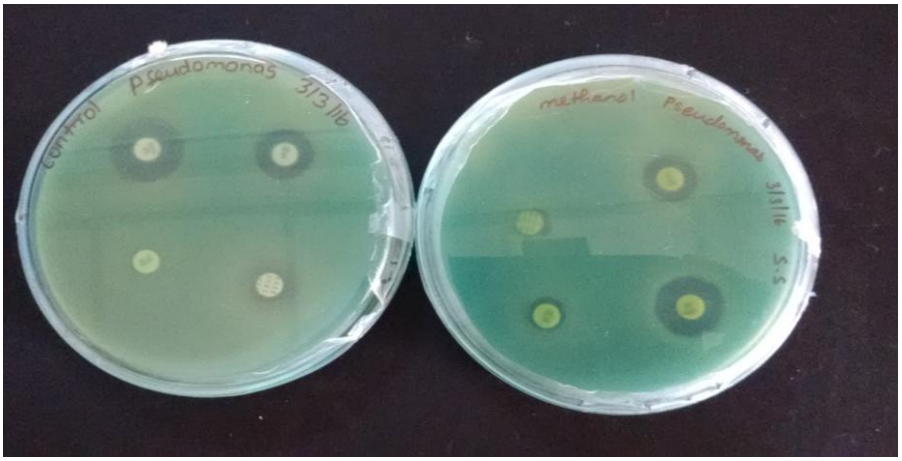

Plate 2: Zone of inhibition of methanol extract in *Pseudomonas sp.*

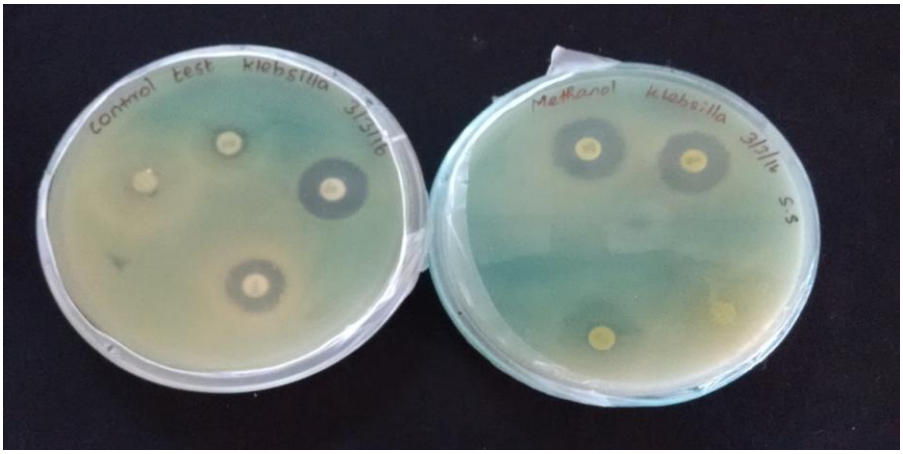

Plate 3: Zone of inhibition of methanol extract in *Klebsilla sp*

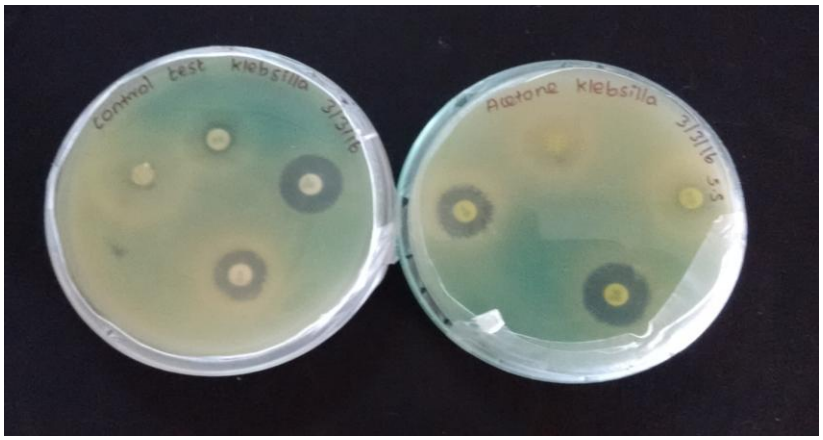

Plate 4: Zone of inhibition of acetone extract in *Klebsiella sp*

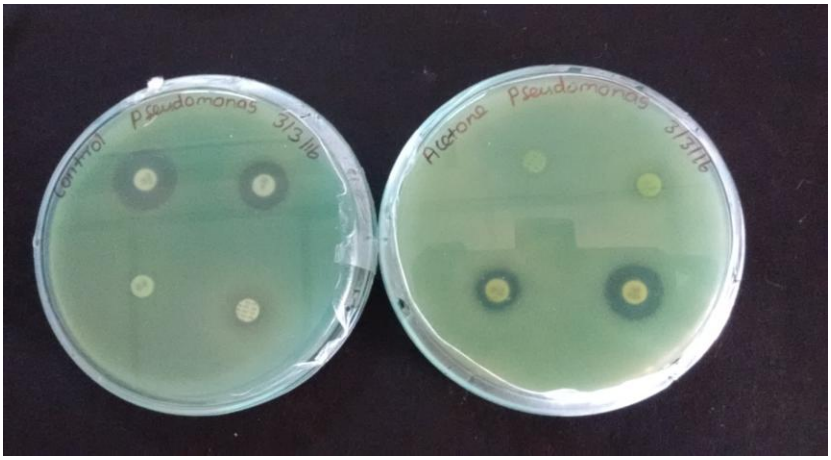

Plate 5: Zone of inhibition of acetone extract in *Pseudomonas sp.*

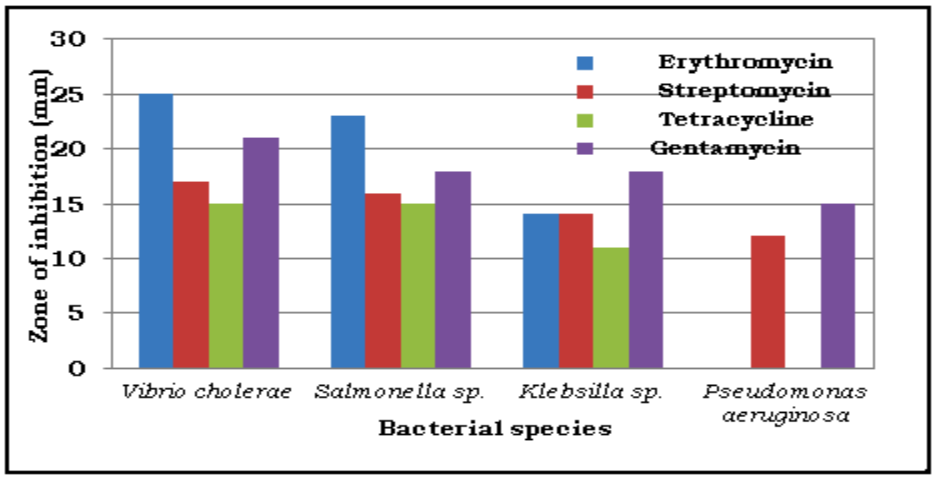

FIGURE 2: Antibiotic sensitivity of pathogenic bacterial strains with plant extracts

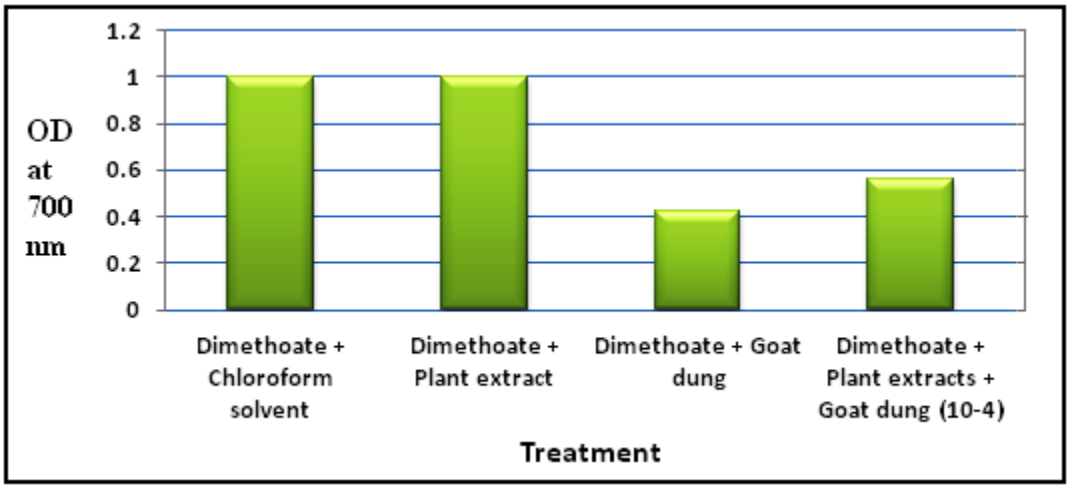

Figure 3 Degradation of Dimethoate in *Prosopis Juliflora* along with Goat Dung

RESULTS:

Quantitative Analysis of Prosopis Juliflora Percentage

The ethanol crude extracts of *Prosopis juliflora* showed high concentration of alkaloids and terphenoids. In which the extracts has flavonoides of 0.5g, terphenoids, phenolics, tannins, alkaloids.(Figure 1).

Antibacterial Activity of Prosopis juliflora

Prosopis juliflora showed better zone of inhibition with 23mm in vibrio cholerea, salmonella and low zone of inhibition with pseudomonas aeruginosa . The zone of inhibition varied according to the rate of dilution.

Degradation of Pesticides

The pesticides were treated with Dimethoate and control tubes are high value. The pesticides, treated with Dimethoate + plant extracts were decreased in compare to the Dimethoate + plant extracts + Goat dung in OD value. (Figure 3).

6.DISCUSSION

The plant based biodegradation is an important factors to protect our environment pollution. In this paper, we have studied degradation of dimethoate in Prosopis *juliflora* along with goat dung pesticides in the soil. For bacterial infections, antibiotics provide therapy for main basis. Thus, there has been a continuing search for new and more potent antibiotics.(12). According to World Health Report of Infectious diseases, by the next decades antibiotic resistance is one of the major issues. In the last decade witnessed to identify the plants as a source of human use (13). *Prosopis juliflora* is used to produce bioactive compounds leads to the development of new pharmaceutical products for their therapeutic use and also have the antibacterial activity.(14) Screening and identification of various natural organic bioactive compounds are very needing for therapeutic use because the successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. Dimethoate is the insecticide which has been widely used all over the world to protect the crop plants from the pests and insects. In the present study, we investigated to find the degradation of Dimethoate by *Prosopis juliflora*. The results show that the antibacterial activity of Prosopis juliflora leaf crude extract showed high zone of inhibition in Salmonella sp. Presence of quantitative estimation of secondary metabolites showed the ethanol crude extract of *Prosopis juliflora* showed high concentrations of alkaloid and terpenoids. Degradation of leaf extracts of *Prosopis juliflora* there is a decrease in concentration of Dimethoate along with goat dung. Dimethoate and leaf extracts with goat dung compare to the Dimethoate and plant extracts to decrease the concentration of the pesticides.

7.CONCLUSION

Prosopis juliflora has proved to be a promising and rare species for the driest zones of the Northeast's Semiaarid Region, occur spontaneously in arid- tropical zones. Leaf degradation showed better in the dimethoate treated comparing to the control.The dimethoate treated leaf with the pesticides treated in better degradation in the less concentrated pesticides. Antibacterial activity of crude leaf extracts showed high zone of inhibition against Vibrio cholera, Salmonella sp. and it has high amount of alkaloids and terpenoids identified by quantitative analysis. The plant uptake and accumulate more concentration of dimethoate in the leaf along with goat dung. The decrease the concentration of dimethoate in plant extracts along with goat dung the degradation of pesticides.

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